

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

Claim 1 (previously presented): An amplification-based method for producing a mammalian promoter-containing siRNA expression cassette, comprising:

i) treating one strand of a double-stranded mammalian promoter sequence, in an amplification reaction mixture, with an oligonucleotide primer which is complementary to the 5' end of the mammalian promoter sequence, wherein the mammalian promoter sequence is capable of transcribing an siRNA molecule in mammalian cells;

ii) treating the other strand of the mammalian promoter sequence, in the amplification reaction mixture, with a second oligonucleotide primer which is complementary to the 3' end of the mammalian promoter sequence, wherein the second primer comprises a sequence which is complementary to a sequence encoding either a sense sequence of an siRNA molecule or an antisense sequence of an siRNA molecule, along with a terminator sequence; and

iii) treating the amplification reaction mixture of steps (i) and (ii) in an amplification reaction at a temperature for annealing and extending said primers on the mammalian promoter sequence and at a temperature for denaturing the extension products to provide an amplified product comprising the mammalian promoter, a sequence encoding either the sense sequence of the siRNA molecule or the antisense sequence of the siRNA molecule, and the terminator sequence, and wherein steps (i)-(iii) are repeated a sufficient number of times to amplify the mammalian promoter-containing siRNA expression cassette.

Claim 2 (original): The method of claim 1, wherein the method is a PCR-based method.

Claim 3 (previously presented): The method of claim 1, wherein the mammalian promoter is a Pol III promoter.

Claim 4 (original): The method of claim 3, wherein the Pol III promoter is a mammalian U6 promoter.

Claim 5 (original): The method of claim 4, wherein the U6 promoter is a human U6 promoter.

Claim 6 (original): The method of claim 1, wherein the sequence encoding the terminator sequence comprises a sequence of about 4-6 deoxyadenosines.

Claim 7 (original): The method of claim 6, wherein the sequence encoding the terminator sequence comprises a sequence of 6 deoxyadenosines.

Claim 8 (original): The method of claim 1, wherein the second primer further comprises a tag sequence to identify functional siRNA encoding sequences.

Claim 9 (original): The method of claim 8, wherein the tag sequence further comprises a restriction site useful for cloning.

Claims 10-16 (canceled).

Claim 17 (previously presented): The method of claim 1, further comprising the step of transfecting a mammalian cell *in vitro* with the amplified mammalian promoter-containing siRNA expression cassette, wherein an siRNA molecule is expressed.

Claim 18 (canceled).

Claim 19 (original): The method of claim 17, wherein one or more of the oligonucleotide primers are modified.

Claim 20 (original): The method of claim 19, wherein one or more of the oligonucleotide primers are modified by phosphorylation.

Claim 21 (original): The method of claim 17, further comprising the step of screening for a target site on mRNA sensitive to the expressed siRNA molecule.

Claim 22 (original): The method of claim 17, wherein the cell is transfected with two or more different siRNA expression cassettes.

Claim 23 (original): The method of claim 22, wherein the different siRNA expression cassettes contain one or both of a different siRNA encoding gene and a different promoter.

Claims 24-32 (canceled).